SCIENCE CHINA
Life Sciences

• RESEARCH PAPERS •

May 2011 Vol.54 No.5: 434–441
doi: 10.1007/s11427-011-4168-8

Characterization of mitochondrial genome of sea cucumber *Stichopus horrens*: A novel gene arrangement in Holothuroidea

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Received January 31, 2011; accepted February 13, 2011

The complete mitochondrial DNA sequence contains useful information for phylogenetic analyses of metazoa. In this study, the complete mitochondrial DNA sequence of sea cucumber *Stichopus horrens* (Holothuroidea: Stichopodidae: *Stichopus*) is presented. The complete sequence was determined using normal and long PCRs. The mitochondrial genome of *Stichopus horrens* is a circular molecule 16257 bps long, composed of 13 protein-coding genes, two ribosomal RNA genes and 22 transfer RNA genes. Most of these genes are coded on the heavy strand except for one protein-coding gene (*nad6*) and five tRNA genes ($tRNA^{Ser(UCN)}$, $tRNA^{Gln}$, $tRNA^{Ala}$, $tRNA^{Val}$, $tRNA^{Asp}$) which are coded on the light strand. The composition of the heavy strand is 30.8% A, 23.7% C, 16.2% G, and 29.3% T bases (AT skew=0.025; GC skew=–0.188). A non-coding region of 675 bp was identified as a putative control region because of its location and AT richness. The intergenic spacers range from 1 to 50 bp in size, totaling 227 bp. A total of 25 overlapping nucleotides, ranging from 1 to 10 bp in size, exist among 11 genes. All 13 protein-coding genes are initiated with an ATG. The TAA codon is used as the stop codon in all the protein coding genes except *nad3* and *nad4* that use TAG as their termination codon. The most frequently used amino acids are Leu (16.29%), Ser (10.34%) and Phe (8.37%). All of the tRNA genes have the potential to fold into typical cloverleaf secondary structures. We also compared the order of the genes in the mitochondrial DNA from the five holothurians that are now available and found a novel gene arrangement in the mitochondrial DNA of *Stichopus horrens*.

complete mitochondrial DNA, *Stichopus horrens*, gene arrangement, Holothuroidea

Citation: Fan S G, Hu C Q, Wen J, *et al*. Characterization of mitochondrial genome of sea cucumber *Stichopus horrens*: A novel gene arrangement in Holothuroidea. Sci China Life Sci, 2011, 54: 434–441, doi: 10.1007/s11427-011-4168-8

With few exceptions, all animal mitochondrial DNAs (mtDNA) contain 37 genes that include 13 protein coding genes (PCGs), two RNA genes (12S RNA and 16S RNA), and 22 tRNA genes necessary for translation of the proteins coded by the mtDNA [1]. Because of its compact size, multiple copy status in a cell, rapid evolutionary rate and lack of recombination, mtDNA has been extensively used as a marker for evolutionary and genetic diversity studies and for the identification of species [2]. Studies into the phylogenetic relationship of metazoans based on mitochondrial genomic sequences have become increasingly popular [3,4]. In addition to mtDNA sequence data, the order of the mito-

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Abbreviations: *atp6* and *8*, ATPase subunits 6 and 8; *cob*, cytochrome b; *cox1–3*, cytochrome c oxidase subunits I–III; *nad1-6* and *4L*, NADH dehydrogenase subunits 1–6 and 4L; *srRNA* and *lrRNA*, small and large subunits ribosomal RNA; *tRNA*, transfer RNA (tRNA) genes; mtDNA: mitochondrial DNA; bp, base pair(s); *L1*, $tRNA^{Leu(CUN)}$; *L2*, $tRNA^{Leu(UUR)}$; *S1*, $tRNA^{Ser(AGN)}$; *S2*, $tRNA^{Ser(UCN)}$.

chondrial genes in the sequence has received extensive attention as a phylogenetic marker [1,5–7]. Major rearrangements have been found in the mtDNA of echinoderms and comparisons of these gene arrangements have great potential for resolving some of the deepest branches of echinoderm phylogeny [5,8–12].

The class Holothuroidea, also known as sea cucumber, is one of five echinoderms. It includes more than 1400 described species around the world [13]. However, the phylogenetic relationships of these holothurians have not been fully resolved [14]. Because of a fundamental assumption that shared gene arrangements imply common ancestry, a comparison of the gene arrangements in holothurians mtDNA may resolve contentious evolutionary relationships [15].

Stichopus horrens is found in the Pacific Ocean from Malaysia to the Society Islands, around French Polynesia, and from southern Japan and Hawaii to New Caledonia [16]. The body of *S. horrens* is grey-brown with irregular grey-white spots [16]. It is cryptic and lethargic by day, and can be found in cracks, caves and crevasses in the rocky substrate at night [17]. It is used as food in China [18].

In this study, we sequenced the complete mitochondrial genome of *Stichopus horrens* and, by comparing *S. horrens* mtDNA with the mtDNA from four other holothurians, found a novel gene arrangement in the Holothuroidea class. The present study contributes new data which could be used for both genomic and evolutionary research on Holothuroidea.

1 Materials and methods

1.1 Sample collection and identification

Adult *S. horrens* were collected subtidally by scuba diving in the Xisha islands, Hainan Province, China. The samples were fixed in 100% ethanol, transported to the laboratory and stored at −20°C. Ossicles were observed by scanning electronic microscopy (SEM). The dorsal body walls were treated with 10% sodium hypochlorite solution for 1–2 min. The digests were then rinsed four times in distilled water, dried, coated with gold-palladium in a sputter coater (Hitachi, E-1010) and examined with a SEM (Hitachi, S-3400N). Species identification was based on the taxonomic descriptions of dermal ossicles by Liao [19] and Massin *et al*. [16].

1.2 DNA extraction

S. horrens DNA was extracted from about 30 mg of sea cucumber muscle tissue using the TIANamp Marine Animals DNA Kit (Tiangen Biotech Co. Ltd., China) and stored at −20°C until required.

1.3 PCR amplification and sequencing

The sequence of the complete mitochondrial genome of *S. horrens* was determined using normal and long PCRs. All PCRs were performed using a PTC-100 thermal cycler (MJ Research, USA). All primers used in this study and their thermal cycling profiles are shown in Table 1. Four short fragments of IrRNA, cox1, cob and cox3 were first amplified using three universal primers, 16sb/coIer [20], cobF424/cobR876 [21], and cox3F/cox3R [21], and 16s1F/16s1R designed in this study (Table 1). The reaction mixtures contained 1 μg of extracted template DNA, 0.2 μmol L^{-1} of each primer, 15 mmol L^{-1} $MgCl_2$, 100 μmol L^{-1} of each dNTP, 2 U of Taq DNA polymerase (TaKaRa, Japan), and ddH_2O to 50 μL. PCR products were checked by electrophoresis on 1% agarose gel, purified using the TIANamp Mini Purification Kit (Tiangen Biotech Co. Ltd., China) and

Table 1 Primers used for amplification of the complete mitochondrial genome of the sea cucumber *Stichopus horrens*[a)]

Order	Primer name	Sequence (5′–3′)	Amplification conditions	Product size (bp)	Source
1*	16s1F 16s1R	GAGACCAGGAAAGGACAATAAGATC TCGGTCTGAACTCAGATCATGTAG	35×(94°C 30 s, 55°C for 30 s, 72°C for 1min)	897	this study
2**	16sb coIer	GACGAGAAGACCCTGTGGAGC GCTCGTGTRTCTACRTCCAT	35×(95°C 30 s, 50°C for 30 s, 72°C for 1.5 min)	1193	[20]
3*	cobF424 cobR876	GGWTAYGTWYTWCCWTGRGGWCARAT GCRTAWGCRAAWARRAARTAYCAYTCWGG	35×(94°C 30 s, 50°C for 30 s, 72°C for 1 min)	449	[21]
4*	cox3F cox3R	TGGTGGCGAGATGTKKTNCGNGA ACWACGTCKACGAAGTGTCARTATCA	35×(94°C 30 s, 45°C for 30 s, 72°C for 1 min)	575	[21]
5*	cox1-3F cox1-3R	GGCATCCAGAAGTTTACATCCT TTGGAGATAGGCTTCTGTGGA	35×(94°C 30 s, 48°C for 30 s, 72°C for 3.5 min)	3161	this study
6*	cox3-cobF cox3-cobR	AGCAGCAGCCTGATACTGACACTTC TCATACTCACTGTACGACGGTTTCTCC	35×(94°C 30 s, 49°C for 30 s, 72°C for 4 min)	4880	this study
7*	cob-16sF cob-16sR	CAACAGGGCTAGACAGAAGATACGAC GGTTTAACGCTTACCGAAATGATGG	35×(94°C 30 s, 46°C for 30 s, 72°C for 5 min)	5855	this study

a) *, The initial step of 94°C for 2 min at the beginning, and final step of 72°C for 10 min after 35 cycles are omitted in the cycling profile of each primer pair in the table. **, The initial steps of 94°C for 1 min, 50°C for 30 s and 72°C for 1.5 min are omitted from the profile in the table.

cloned into PMD18-T vectors (TaKaRa, Japan). The resultant plasmid was sequenced using the versatile primer M13-47F/M13- 47R on ABI 3730 (ABI, USA).

The whole mitochondrial genome was then amplified using a long PCR technique [22]. Three pairs of primers, cox1-3F/cox1-3R, cox3-cobF/cox3-cobR and cob-16sF/cob-16sR, were designed based on the obtained partial sequences and the entire mitochondrial genome was amplified (Table 1). The reactions were carried out in a final volume of 50 μL containing 15 mmol L^{-1} $MgCl_2$, 0.2 μmol L^{-1} of each primer, 200 μmol L^{-1} of each dNTP, 1 μg of template DNA and 2.5 U of LA Taq DNA polymerase (TaKaRa, Japan). All PCR products were sequenced directly on ABI 3730 (ABI, USA) using primer walking.

1.4 Sequence analysis

All the obtained sequences were confirmed using the NCBI BLAST search and assembled using SeqMan software (DNAStar, Madison, USA). PCGs and ribosomal RNA genes were determined by aligning the sequences with the *Apostichopus japonicus* mitochondrial genome [12]. Transfer RNAs were identified using tRNAscan-SE software (version 1.21) [23] in the 'default' search mode, using the echinoderm mitochondrial genetic code, 'mito/chloroplast' source and by setting the cove cutoff score to 1 when necessary. Nucleotide composition and code usage were estimated with the MEGA4.0 program [24]. Composition skewness was calculated according to the formulas: GC skew=(G%–C%)/(G%+C%), AT skew=(A%–T%)/(A%+T%) [25]. The gene map of the mitochondrial genome was created with OGDRAW v1.1 [26] and modified manually.

1.5 Analysis of the mtDNA gene order

The complete mtDNA sequences of four other holothurians, *Holothuria forskali* (FN562582), *Apostichopus japonicus* (EU294194) [12], *Parastichopus nigripunctatus* (AB525762) and *Cucumaria miniata* (NC_005929) [27], are currently available in GenBank. These along with the complete mtDNA sequence of *S. horrens* were used to analyze the arrangement of the mtDNA genes.

2 Results and discussion

2.1 Species identification

Two different kinds of table ossicles (TB1 and TB2), C-shaped rods and rosettes were observed in the dorsal body walls of *S. horrens* (Figure 1). The size, structure and shape of these ossicles were in agreement with previous studies [16,19]. The tack-like table ossicles, TB1s, are 110–130 μm across. TB2s (30–35 μm across) are smooth, perforated by 4 large central holes and 4–10 small peripheral holes. The C-shaped rods are 20–50 μm long and the rosettes are 17–30 μm long. *S. horrens* is readily identified by the presence of the diagnostic distinct tack-like table ossicles (TB1) (Figure 1A) [16,28].

2.2 Mitochondrial genome organization

The complete mtDNA sequence of *S. horrens* was determined using normal PCR and long PCR. As found in other metazoans, the DNA is a circular double helix, 16257 bp in length with 13 PCGs, 22 transfer RNAs and 2 ribosomal RNA genes (Figure 2 and Table 2) [1]. The size of *S. horrens* mtDNA is within the size range of holothurian mtDNA genomes sequenced to date, i.e. from 15841 bp (*Holothuria forskali*) to 17538 bp (*Cucumaria miniata*) (Table 3). Except for one PCG (*nad6*) and five tRNA genes ($tRNA^{Ser(UCN)}$, $tRNA^{Gln}$, $tRNA^{Ala}$, $tRNA^{Val}$, $tRNA^{Asp}$) coded for on the light (L)-strand, all the other genes were coded for on the heavy (H)-strand (Figure 2 and Table 2). The overall base composition of the H-strand was estimated to be 30.8% A, 23.7% C, 16.2% G, and 29.3% T. The G+C content of *S. horrens* mtDNA is 39.9%, higher than that of *A. japonicus/P. nigripunctatus* (38.1%), *H. forskali* (37.8%) and *C. miniata* (36.2%) (Table 3). The AT skew for the 5 holothurian mtDNAs is positive (0.010 to 0.119), indicating a slight bias toward A rather than T (Table 3). The GC skew for the 5 holothurian mtDNAs is negative (–0.271 to –0.055), indicating the occurrence of more Cs than Gs (Table 3). The entire mtDNA sequence of *S. horrens* has been deposited in GenBank (accession number: HQ000092).

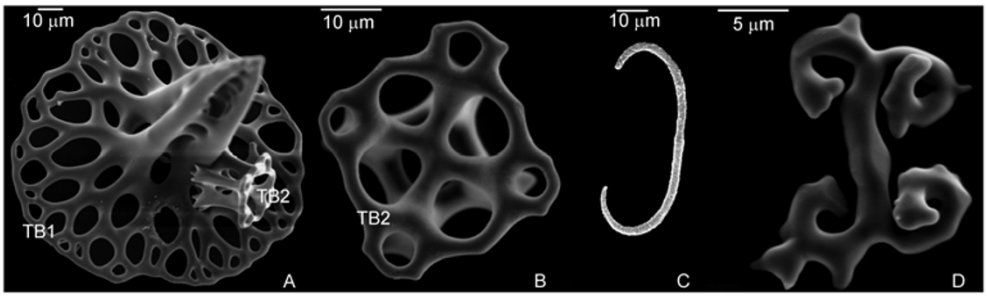

Figure 1 Dorsal ossicles of *Stichopus horrens*. A, TB1 and TB2; B, TB2; C, C-shape rod; D, rosettes.

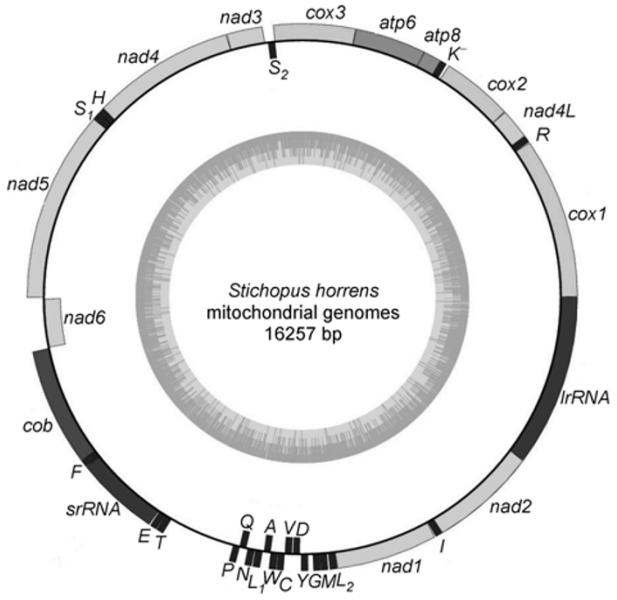

Figure 2 Gene map of the mitochondrial genome of *Stichopus horrens*. Genes coded on the heavy strand are on the outside of the circular gene map and genes coded on the light strand are on the inside. The inner ring displays the GC content.

2.3 Protein-coding genes

Together, the 13 mitochondrial PCGs in *S. horrens* are 11397 bp in length, longer than the PCGs in the other four holothurians (Table 3). The longest PCG in *S. horrens* is *nad5* (1845 bp) and the shortest is *atp8* (177 bp) (Table 2). Six reading frame overlaps were found within the mtDNA of *S. horrens*: *nad4* and $tRNA^{His}$ share 10 nucleotides; *atp8* and *atp6* share 7 nucleotides; *cox3* and $tRNA^{Ser(UCN)}$ share 2 nucleotides; and $tRNA^{Lys}$ and *atp8,* and $tRNA^{Leu(UUR)}$ and *nad1* share 1 nucleotide each (Table 2). The initiation codon for all 13 PCGs is ATG (Table 2). Two genes *nad3* and *nad4* use TAG as the termination codon; the others, *atp6*, *atp8*, *cob*, *cox1-3*, *nad1-2*, *nad4L* and *nad5-6*, all use TAA as the stop codon (Table 2). There are a total of 3799 codons in the 13 mitochondrial PCGs. The most frequently used amino acids are Leu (16.29%), Ser (10.34%), Ile (9.61%) and Phe (8.37%) (Table 4). The highest GC content among the 13 PCGs of *S. horrens* mtDNA is in *nad3* (43.7%) and the lowest is in *atp8* (32.8%) (Table 5). All the PCGs have a small GC skew of −0.399 to −0.105. Four PCGs, *atp8*, *nad4*, *nad5* and *nad6*, have a slight positive AT skew while the other nine PCGs have a negative AT skew (Table 5).

Table 2 Annotation of the mitochondrial genome of *Stichopus horrens*[a)]

Gene	Strand	Sequence location	Size (bp)	Start codon	Stop codon	Anticodon	Intergenic region*
cox1	+	1–1554	1554	ATG	TAA		5
$tRNA^{Arg}$	+	1560–1627	68			TCG	0
nad4L	+	1628–1924	297	ATG	TAA		0
cox2	+	1925–2614	690	ATG	TAA		20
$tRNA^{Lys}$	+	2635–2699	65			CTT	–1
atp8	+	2699–2875	177	ATG	TAA		–7
atp6	+	2869–3552	684	ATG	TAA		2
cox3	+	3555–4337	783	ATG	TAA		–2
$tRNA^{Ser(UCN)}$	–	4336–4406	71			TGA	32
nad3	+	4439–4783	345	ATG	TAG		4
nad4	+	4788–6158	1371	ATG	TAG		–10
$tRNA^{His}$	+	6149–6216	68			GTG	2
$tRNA^{Ser(AGN)}$	+	6219–6286	68			GCT	0
nad5	+	6287–8131	1845	ATG	TAA		12
nad6	–	8144–8632	489	ATG	TAA		8
cob	+	8641–9783	1143	ATG	TAA		0
$tRNA^{Phe}$	+	9784–9854	71			GAA	0
srRNA	+	9855–10674	820				0
$tRNA^{Glu}$	+	10675–10743	69			TTC	3
$tRNA^{Thr}$	+	10747–10816	70			TGT	0
Putative control region	+	10817–11491	675				0
$tRNA^{Pro}$	+	11492–11558	67			TGG	–4
$tRNA^{Gln}$	–	11555–11624	70			TTG	17
$tRNA^{Asn}$	+	11642–11710	69			GTT	8
$tRNA^{Leu(CUN)}$	+	11719–11790	72			TAG	14
$tRNA^{Ala}$	–	11805–11872	68			TGC	1
$tRNA^{Trp}$	+	11874–11943	70			TCA	3

(*To be continued on the next page*)

(*Continued*)

Gene	Strand	Sequence location	Size (bp)	Start codon	Stop codon	Anticodon	Intergenic region*
$tRNA^{Cys}$	+	11947–12013	67			GCA	1
$tRNA^{Val}$	–	12015–12086	72			TAC	11
$tRNA^{Asp}$	–	12098–12165	68			GTC	13
$tRNA^{Tyr}$	+	12179–12246	68			GTA	50
$tRNA^{Gly}$	+	12297–12366	70			TCC	2
$tRNA^{Met}$	+	12369–12437	69			CAT	12
$tRNA^{Leu(UUR)}$	+	12450–12522	73			TAA	–1
nad1	+	12522–13493	972	ATG	TAA		7
$tRNA^{Ile}$	+	13501–13568	68			GAT	0
nad2	+	13569–14615	1047	ATG	TAA		0
IrRNA	+	14616–16257	1642				0

a) *, The numbers indicate the number of nucleotides separating the different genes. Negative numbers represent overlapping nucleotides.

Table 3 Size, composition and skew in five holothurian mitochondrial genomes

Species	Size (bp)	A%	T%	G%	C%	GC%	AT skew	GC skew
Whole genome								
H. forskali	15841	31.4	30.8	16.4	21.4	37.8	0.010	–0.132
P. nigripunctatus	16122	31.7	30.1	18.0	20.1	38.1	0.026	–0.055
A. japonicus	16096	31.8	30.1	17.9	20.2	38.1	0.027	–0.060
S. horrens	16257	30.8	29.3	16.2	23.7	39.9	0.025	–0.188
C. miniata	17538	35.7	28.1	13.2	23.0	36.2	0.119	–0.271
Protein-coding genes								
H. forskali	11365	29.0	33.1	16.2	21.6	37.8	-0.066	–0.143
P. nigripunctatus	11379	29.0	32.4	18.1	20.5	38.6	-0.055	–0.062
A. japonicus	11379	29.1	32.3	18.0	20.6	38.6	-0.052	–0.067
S. horrens	11397	28.3	31.5	16.0	24.2	40.2	-0.054	–0.204
C. miniata	11339	32.7	29.6	14.0	23.7	37.7	0.050	–0.257
tRNA								
H. forskali	1513	33.0	27.7	19.6	19.7	39.3	0.087	–0.003
P. nigripunctatus	1518	31.9	30.1	19.0	19.0	38.0	0.029	0.000
A. japonicus	1518	32.1	30.1	18.8	19.0	37.8	0.032	–0.005
S. horrens	1521	33.1	29.7	18.7	18.5	37.2	0.054	0.005
C. miniata	1506	34.3	31	16.8	17.9	34.7	0.051	–0.032
rRNA								
H. forskali	2407	36.6	24.8	18.7	19.9	38.6	0.192	–0.031
P. nigripunctatus	2390	37.2	24.4	20.5	17.9	38.4	0.208	0.068
A. japonicus	2387	37.8	24.3	19.8	18.1	37.9	0.217	0.045
S. horrens	2462	35.9	24.3	18.6	21.2	39.8	0.193	–0.065
C. miniata	2200	41.2	23.9	15.5	19.5	35.0	0.266	–0.114

Table 4 Codon usage in the 13 protein-coding genes of the *Stichopus horrens* mitochondrial genome

Amino acid	Codon	Number	Frequency (%)	Amino acid	Codon	Number	Frequency (%)
Phe	TTT	187	4.92	Tyr	TAT	43	1.13
	TTC	131	3.45		TAC	64	1.68
Leu	TTA	132	3.47	Term	TAA	11	0.29
	TTG	44	1.16		TAG	2	0.05
Leu	CTT	145	3.82	His	CAT	36	0.95
	CTC	111	2.92		CAC	54	1.42
	CTA	148	3.90	Gln	CAA	66	1.74
	CTG	39	1.03		CAG	19	0.50
Ile	ATT	130	3.42	Asn	AAT	36	0.95
	ATC	83	2.18		AAC	53	1.40

(*To be continued on the next page*)

(*Continued*)

Amino acid	Codon	Number	Frequency (%)	Amino acid	Codon	Number	Frequency (%)
	ATA	155	4.08		AAA	116	3.05
Met	ATG	86	2.26	Lys	AAG	57	1.50
Val	GTT	59	1.55	Asp	GAT	21	0.55
	GTC	41	1.08		GAC	50	1.32
	GTA	92	2.42	Glu	GAA	80	2.11
	GTG	18	0.47		GAG	15	0.39
Ser	TCT	70	1.84	Cys	TGT	22	0.58
	TCC	92	2.42		TGC	19	0.50
	TCA	80	2.11	Trp	TGA	78	2.05
	TCG	19	0.50		TGG	20	0.53
Pro	CCT	51	1.34	Arg	CGT	8	0.21
	CCC	41	1.08		CGC	6	0.16
	CCA	68	1.79		CGA	44	1.16
	CCG	15	0.39		CGG	16	0.42
Thr	ACT	62	1.63	Ser	AGT	13	0.34
	ACC	71	1.87		AGC	25	0.66
	ACA	86	2.26		AGA	91	2.40
	ACG	16	0.42		AGG	3	0.08
Ala	GCT	48	1.26	Gly	GGT	41	1.08
	GCC	97	2.55		GGC	29	0.76
	GCA	81	2.13		GGA	97	2.55
	GCG	25	0.66		GGG	41	1.08

Table 5 Base composition and skew for the protein-coding genes found in the mitochondrial genome of *Stichopus horrens*

Gene	Length (bp)	Proportion of nucleotides (%)					AT skew	GC skew
		A	C	G	T	GC		
cox1	1554	25.8	23.1	18.7	32.4	41.8	–0.113	–0.105
nad4L	297	28.3	24.2	10.4	37.0	34.6	–0.133	–0.399
cox2	690	30.0	24.3	14.9	30.7	39.2	–0.012	–0.240
atp8	177	35.6	20.9	11.9	31.6	32.8	0.060	–0.274
atp6	684	29.7	24	13.5	32.9	37.5	–0.051	–0.280
cox3	783	27.6	23.9	18.4	30.1	42.3	–0.043	–0.130
nad3	345	23.8	30.1	13.6	32.5	43.7	–0.155	–0.378
nad4	1371	31.7	24.2	13.6	30.5	37.8	0.019	–0.280
nad5	1845	33.2	24.9	14.5	27.4	39.4	0.096	–0.264
nad6	489	44.6	25.6	15.1	14.7	40.7	0.504	–0.258
cob	1143	28.0	26.6	16.1	29.3	42.7	–0.023	–0.246
nad1	972	25.8	23.9	17.8	32.5	41.7	–0.115	–0.146
nad2	1047	27.2	25.1	15.1	32.6	40.2	–0.090	–0.249
Mean	877	30.1	24.7	15.3	14.9	39.6		

2.4 Transfer and ribosomal RNA genes

22 tRNA genes were found in the mtDNA of *S. horrens* and their lengths ranged from 65 bp ($tRNA^{Lys}$) to 73 bp ($tRNA^{Leu(UUR)}$). As reported in an earlier study of *A. japonicus* mtDNA, $tRNA^{Pro}$ overlapped with $tRNA^{Gln}$ by 4 nucleotides (Table 2) [12]. All tRNA gene sequences can potentially fold into typical clover-leaf secondary structures. The G+C content of the tRNAs is 37.2%, within the range observed for other holothurians (Table 3).

An NCBI BLAST search found matches to the *lrRNA* and *srRNA* genes in the *S. horrens* mtDNA genome. These two rRNAs are coded for on the H-strand. The *S. horrens* homolog of the *lrRNA* gene is 1642 bp in length and lies between the PCGs *nad2* and *cox1*. The *S. horrens* homolog of the *srRNA* gene is 820 bp long and flanked by $tRNA^{Phe}$ and $tRNA^{Glu}$. The G+C content (39.8%) of the *S. horrens rRNA* genes is highest of the five holothurians.

2.5 Non-coding regions

The total length of the non-coding regions of the *S. horrens* mtDNA was found to be 902 bp. The largest continuous non-coding region (675 bp) is located between $tRNA^{Thr}$ and

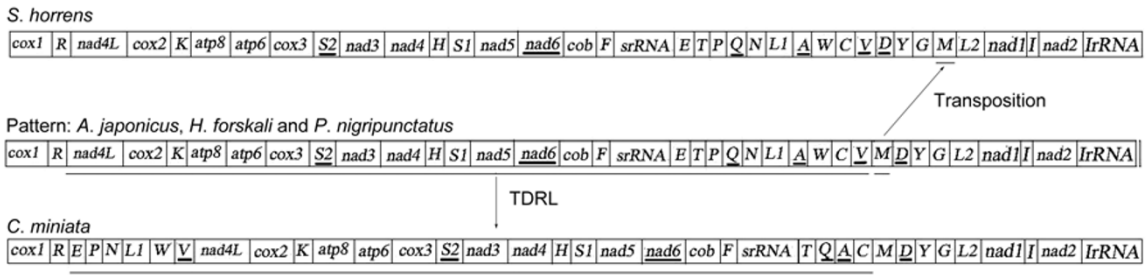

Figure 3 Linearized representation of the conserved mitochondrial gene order for five holothurians. All genes are coded for on the H-strand except those that are underlined; they are coded for on the L-strand. Gene segments are not drawn to scale.

tRNA^{Pro} (Table 2). Because this region is AT-rich (AT%= 58.1) and located in a region that is similar to the control region of *A. japonicus* mtDNA [12], this region is thought to be a putative control region in the mtDNA of *S. horrens*. The second largest non-coding region (50 bp, AT%=60) is located between the *tRNA*^{Tyr} and *tRNA*^{Gly} genes.

2.6 Gene rearrangements

Mitochondrial gene arrangement comparison is a powerful tool for phylogenetic studies [15]. During the past decade, some research on mitochondrial gene orders in echinoderms has been reported [1,5,8,11,12]. Shared gene arrangements likely indicate common ancestry [11]. The mechanisms of genome rearrangement include inversion, transposition, reverse transposition and tandem duplication random loss (TDRL) [8].

In this study, five currently available holothurian mitochondrial genomes were used to analyze mtDNA gene arrangements. The order of the genes on the mitochondrial genomes of *A. japonicus*, *P. nigripunctatus* and *H. forskali* is identical. The arrangement of genes in these genomes was used as the reference in this study (Figure 3). When compared with this pattern, a single TDRL event was found in *C. miniata* mtDNA [5] and a transposition event which moved *tRNA*^{Met} (gene M) from the 3′ end of *tRNA*^{Val} (gene V) to the 3′ end of *tRNA*^{Gly} (gene G) was found in *S. horrens* mtDNA (Figure 3). These results confirm earlier reports that tRNA genes may be among the most mobile elements in the mtDNA genome. This novel arrangement of Holothuroidea mitochondrial genes is reported here for the first time. The order of 21 echinoderm mitochondrial genes was compared and described by Shen *et al*. [12]. The gene arrangement of *S. horrens* mtDNA is also unique among the reported arrangement of echinoderm genes.

Another complete sea cucumber mitochondrial genome from the genus *Stichopus* was also available to us. We found that the mitochondrial gene order of the *Stichopus* was identical to that of *S. horrens* (date not shown). Thus, we propose that this mitochondrial gene arrangement is a feature of sea cucumbers belonging to the genus *Stichopus*. More sea cucumber mtDNA sequences are needed to prove this supposition.

3 Conclusion

In this study we presented the complete mtDNA sequence of the sea cucumber *Stichopus horrens* and found a novel gene arrangement in the Holothuroidea mitochondrial genome. Our results provide basic information required for the phylogenetic analyses of the holothurians that can also be applied to the echinoderms.

This work was supported by the National Key Technologies R&D Program (Grant No. 2009BAB44B02) and the Science and Technology Program of Guangdong Province (Grant Nos. A200901E01, A200899E02 and 2009B091300155).

1 Boore J. Animal mitochondrial genomes. Nucl Acids Res, 1999, 27: 1767–1780
2 Curole J, Kocher T. Mitogenomics: digging deeper with complete mitochondrial genomes. Trends Ecol Evol, 1999, 14: 394–398
3 Cohen B, Ameziane N E, leaume M, *et al*. Crinoid phylogeny: a preliminary analysis (Echinodermata: Crinoidea). Mar Biol, 2004, 144: 605–617
4 Kerr A, Janies D A, Clouse R M, *et al*. Molecular phylogeny of coral-reef sea cucumbers (Holothuriidae: Aspidochirotida) based on 16S mitochondrial ribosomal DNA sequence. Mar Biotechnol, 2005, 7: 53–60
5 Arndt A, Smith M. Mitochondrial gene rearrangement in the sea cucumber genus Cucumaria. Mole Biol Evol, 1998, 15: 1009–1016
6 Ren Z M, Zhu B, Ma E, *et al*. Complete nucleotide sequence and gene arrangement of the mitochondrial genome of the crab-eating frog *Fejervarya cancrivora* and evolutionary implications. Gene, 2009, 441: 148–155
7 Sankoff D, Leduc G, Antoine N, *et al*. Gene order comparisons for phylogenetic inference: Evolution of the mitochondrial genome. Proc Natl Acad Sci USA, 1992, 89: 6575–6579
8 Perseke M, Fritzsch G, Ramsch K, *et al*. Evolution of mitochondrial gene orders in echinoderms. Mol Phylogenet Evol, 2008, 47: 855–864
9 Smith M, Arndt A, Gorski S, *et al*. The phylogeny of echinoderm classes based on mitochondrial gene arrangements. J Mol Evol, 1993, 36: 545–554
10 Smith M, Banfield D K, Doteval K, *et al*. Gene arrangement in sea star mitochondrial DNA demonstrates a major inversion event during echinoderm evolution. Gene, 1989. 76: 181–185
11 Scouras A, Smith M J. A novel mitochondrial gene order in the crinoid echinoderm *Florometra serratissima*. Mole Biol Evol, 2001, 18: 61–73
12 Shen X, Tian M, Liu Z H, *et al*. Complete mitochondrial genome of the sea cucumber *Apostichopus japonicus* (Echinodermata: Holothuroidea): The first representative from the subclass Aspidochirotacea with the echinoderm ground pattern. Gene, 2009, 439: 79–86

13 Smiley S. Holothuroidea. In: Microscopic Anatomy of Invertebrates, Vol 14, Echinodermata, Harrison F W, Chia F S, eds. New York: Wiley-Liss, 1994. 401–471
14 Lacey K, McCormack G P, Keegan B F, *et al*. Phylogenetic relationships within the class holothuroidea, inferred from 18S rRNA gene data. Mar Biol, 2005, 147: 1149–1154
15 Boore J, Brown W. Big trees from little genomes: mitochondrial gene order as a phylogenetic tool. Curr Opin Genet Dev, 1998, 8: 668–674
16 Massin C, Zulfigar Y, Hwai T S, *et al*. The genus *Stichopus* (Echi-nodermata: Holothuroidea) from the Johore Marine Park (Malaysia) with the description of two new species. Bulletin de l'Institut Royal des Sciences Naturelles de Belgique. Biologie, 2002, 72: 73–99
17 Hearn A, Pinillos F, Baseline information on the warty sea cucumber *Stichopus horrens* in Santa Cruz, Galapagos, prior to the commencement of an illegal fishery. Beche-de-Mer, 2006, 24: 3–10
18 Chen J. Overview of sea cucumber farming and sea ranching practices in China. Beche-de-mer, 2003, 18: 18–23
19 Liao Y L. Fauna Sinica Phylum Echinodermata Class Holothuroidea. Beijing: Science Press, 1997
20 Arndt A, Marquez C, Lambert P, *et al*. Molecular phylogeny of eastern Pacific sea cucumbers (Echinodermata: Holothuroidea) based on mitochondrial DNA sequence. Mol Phylogenet Evol, 1996, 6: 425–437
21 Boore J, Macey J, Medina M. Sequencing and comparing whole mitochondrial genomes of animals. Methods Enzymol, 2005, 395: 311–348
22 Cheng S, Chang S Y, Gravitt P, *et al*. long PCR. Nature, 1994, 23: 685–685
23 Lowe T M, Eddy S R. tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. Nucl Acids Res, 1997, 25: 955–964
24 Tamura K, Dudley J, Nei M, *et al*. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mole Biol Evol, 2007, 24: 1596–1599
25 Perna N, Kocher T. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. J Mol Evol, 1995, 41: 353–358
26 Lohse M, Drechsel O, Bock R. OrganellarGenomeDRAW (OGDRAW): a tool for the easy generation of high-quality custom graphical maps of plastid and mitochondrial genomes. Curr Genet, 2007, 52: 267–274
27 Scouras A, Beckenbach K, Arndt A, *et al.* Complete mitochondrial genome DNA sequence for two ophiuroids and a holothuroid: the utility of protein gene sequence and gene maps in the analyses of deep deuterostome phylogeny. Mol Phylogenet Evol, 2004, 31: 50–65
28 Byrne M, Rowe F, Uthicke S. Molecular taxonomy, phylogeny and evolution in the family Stichopodidae (Aspidochirotida: Holothuroidea) based on COI and 16S mitochondrial DNA. Mol Phylogenet Evol, 2010, 3: 1068–1081